Porcine Epidemic Diarrhea (PED)

Makoto Yamakawa, DVM, PhD

National Institute of Animal Health
National Agriculture and Food Research Organization (NARO)
Japan
PED has been reported in Asia including Japan. Suckling pigs have been seriously damaged in Asian countries.

PR of China:
1st case was recognized in 1973. PEDV was identified in 1984. Affected cases reached about 40,000 in 2005. It is reported that over 1 million piglets died in the large-scale outbreaks after 2010. PED is a disease endemic to the country. PEDV was detected 79.7% of farms (141/177) in 29 provinces by molecular epidemiological investigation performed from Feb.2011 to Nov.2012. Comparison of the S gene sequences of 33 isolates indicates that 13 strains belong to the traditional group and 20 strains belong to the new group. The latter group is now prevalent in many places around the country.

Republic of Korea:
Suspected cases of PED were found frequently in 1980’s. PEDV was isolated in 1992 and the large-scale outbreaks were reported in 1990’s. PED is a disease endemic to the country. Occurrence of PED has been increasing after Nov. 2013.

Chinese Taipei:
PED has been reported mainly middle and southern areas after Jan. 2014.

Thailand, Vietnam and Philippines:
Large-scale outbreaks were observed between 2006 and 2008.
Occurrence of PED in Japan

PED is one of the notifiable diseases under the Act on Domestic Animal Infectious Diseases Control (after 1997).

PED has been recognized from 1980’s, but most of the cases are sporadic with the exception of an outbreak in 1996 (Affected: approx. 80,000, Dead: 39,539).

No outbreaks of PED were reported from 2007 to 2012. Neutralizing antibody prevalence rate against PEDV has been kept under 4.0% in recent ten years (0.3-3.8%).

In 2013, a large-scale outbreak was confirmed again after the absence of 7 years.
In last decade, pig population of Japan hovers below the 10 million.

Pig farms are clustered close together in these areas.

Miyazaki; Outbreak of FMD in 2010

Distribution of pig population

Heads
- >500,000
- 100,000-<500,000
- 10,000-<100,000
- <10,000

Joint FAO/OIE Workshop on Swine Disease Control in Asia
PED case reports by week

Cases

Joint FAO/OIE Workshop on Swine Disease Control in Asia
### Occurrence of PED in Japan 2013-2014

<table>
<thead>
<tr>
<th>Region</th>
<th>Cases</th>
<th>Dead pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hokkaido</td>
<td>23</td>
<td>14,275</td>
</tr>
<tr>
<td>Tohoku</td>
<td>81</td>
<td>56,397</td>
</tr>
<tr>
<td>Kanto</td>
<td>225</td>
<td>127,934</td>
</tr>
<tr>
<td>Chubu</td>
<td>140</td>
<td>45,578</td>
</tr>
<tr>
<td>Kinki</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chugoku/Shikoku</td>
<td>19</td>
<td>5,863</td>
</tr>
<tr>
<td>Kyusyu/Okinawa</td>
<td>329</td>
<td>122,895</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>817</td>
<td>372,942</td>
</tr>
</tbody>
</table>

**Notes:**
- Affected prefectures: 38/47 (80.9%)
- Affected farms: 817/5,570 (14.7%)
- Affected pigs: approx. 1,227,000/9,685,000 (12.7%)
- Dead pigs: approx. 373,000/9,685,000 (3.9%)
- (Almost all piglets: 27% of affected pigs died)

From October 1, 2013 to Aug. 31, 2014

As of August 31, 2014, pig farms in these areas are clustered close together.
Diagnosis of PED

- Epidemiological investigation
- Clinical findings
  - Pathological findings
  - Postmortem examination
  - Diarrheal feces

Histological diagnosis
- HE stain
- IHC using anti-PEDV Rabbit serum

Virological diagnosis
- IFA (antigen detection)
- Virus isolation
- Gene detection by RT-PCR (real-time RT-PCR)
- Sequencing
  - Discrimination between PED and TGE

Serological diagnosis (Neutralization test)

Finally identify PED by using these data
Characterization of the strain of PED virus prevailing in recent years

Physicochemical, genetic and antigenic analyses of the new strains
Clinical, pathological and epidemiological investigations

Isolation of virus from infected pigs in the field
Determination of the nucleotide sequence of isolates
Antigenic comparison among the vaccine strain, traditional and recent isolates
Serological surveillance
Experimental infection of pigs using isolates
Confirmation of the efficacy of disinfectants
Improvement and development of diagnostic and preventive methods

Establishment of more effective measures for control and prevention of PED
**Phylogenetic analysis of the spike(S1) region**

PEDV strains are genetically segregated into two groups. Sequence identities of these strains vary from 93 to 100%. PEDV is serologically monotypic.

- **nucleotide sequences** -

**North American Type**
- Japan (2013-2014)
- USA (2013-2014)
- Korea (2013-2014)
- China (2011-2013)

**Japanese Classical (90’s) & Vaccine strains**
- INDELS Type

**Japanese Classical (80’s) & Vaccine strains**
- Group I
- Group II

PEDV strains are genetically segregated into two groups. Sequence identities of these strains vary from 93 to 100%. PEDV is serologically monotypic.

**Spike**
Neutralizing antigen

**North American Type**
- Japan (2013-2014)
- USA (2013-2014)
- Korea (2013-2014)
- China (2011-2013)

**Japanese Classical (90’s) & Vaccine strains**
- INDELS Type

**Japanese Classical (80’s) & Vaccine strains**
- Group I
- Group II

PEDV strains are genetically segregated into two groups. Sequence identities of these strains vary from 93 to 100%. PEDV is serologically monotypic.

**Spike**
Neutralizing antigen
**Alignment of the N-terminus residues of the S protein**

<table>
<thead>
<tr>
<th>CH851</th>
<th>MKSLNYFWLFLPVLSTLSPQDVTRCOSTINI FRRFFSKFNVOAPAVVVLGGYLPMSN</th>
<th>SWYGCTGLETASGVHGIFLSYYIDAGQG</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH/HBQX/10</td>
<td>T I L P T</td>
<td></td>
</tr>
<tr>
<td>JPN2014-5</td>
<td>T S A N T</td>
<td></td>
</tr>
<tr>
<td>JPN2014-6</td>
<td>T S A N T</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IA1</th>
<th>T S A N T</th>
<th>I GENQGVNSTT A G Q H P</th>
<th>V H R G H</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH2012</td>
<td>T S A N T</td>
<td>I GENQGVNSTT A G Q H P</td>
<td>V H R G H</td>
</tr>
</tbody>
</table>

| JPN2013-1 | T S A N T | I GENQGVNSTT A G Q H P | V H R G H |
| JPN2013-2 | T S A N T | I GENQGVNSTT A G Q H P | V H R G H |
| JPN2013-3 | T S A N T | I GENQGVNSTT A G Q H P | V H R G H |
| JPN2013-4 | T S A N T | I GENQGVNSTT A G Q H P | V H R G H |
| JPN2014-1 | T S A N T | I GENQGVNSTT A G Q H P | V H R G H |
| JPN2014-2 | T S A N T | I GENQGVNSTT A G Q H P | V H R G H |
| JPN2014-3 | T S A N T | I GENQGVNSTT A G Q H P | V H R G H |
| JPN2014-4 | T S A N T | I GENQGVNSTT A G Q H P | V H R G H |

<table>
<thead>
<tr>
<th>OH851</th>
<th>FEIGISQEPFDPSGYQLHCLKATNGNHNAIARLRI QCPDNKTLGPTVN</th>
<th>DVTGRNCLFNNIKAI PAYMQDGGKN</th>
<th>VVGITWDNDVRVTVFAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV777</td>
<td>T I L P</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>IA1</td>
<td>T T S I A N</td>
<td>S I A N</td>
<td>H SEHS</td>
</tr>
<tr>
<td>AH2012</td>
<td>T T S I A N</td>
<td>S I A N</td>
<td>H SEHS</td>
</tr>
<tr>
<td>JPN2013-1</td>
<td>T T S I A N</td>
<td>S I A N</td>
<td>K S</td>
</tr>
<tr>
<td>JPN2013-2</td>
<td>T T S I A N</td>
<td>S I A N</td>
<td>K S</td>
</tr>
<tr>
<td>JPN2013-3</td>
<td>T T S I A N</td>
<td>S I A N</td>
<td>K S</td>
</tr>
<tr>
<td>JPN2013-4</td>
<td>T T S I A N</td>
<td>S I A N</td>
<td>K S</td>
</tr>
<tr>
<td>JPN2014-1</td>
<td>T T S I A N</td>
<td>S I A N</td>
<td>H SEHS</td>
</tr>
<tr>
<td>JPN2014-2</td>
<td>T T S I A N</td>
<td>S I A N</td>
<td>H SEHS</td>
</tr>
<tr>
<td>JPN2014-3</td>
<td>T T S I A N</td>
<td>S I A N</td>
<td>H SEHS</td>
</tr>
<tr>
<td>JPN2014-4</td>
<td>S Y T T S I A N</td>
<td>S I A N</td>
<td>H SEHS</td>
</tr>
</tbody>
</table>

**INDELS Type**

**Insertion and deletion sites were conserved**

Alignment of the deduced amino acid sequences corresponding to the first 180 N-terminus residues of the S protein of the current Japanese field PEDV strains with those of the US (OH851 and IA1), Chinese (CH/HBQX/10 and AH2012) and the prototype CV777 strains. Dots indicate the amino acids that are identical to those in the strain OH851. Dashes indicate the deleted sequences. Deletions and insertions are boxed.
### Antigenic comparison among PEDV strains

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>CV777</th>
<th>Japanese Classical strain</th>
<th>Japanese 2013 strain</th>
<th>Japanese INDELS strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV777</td>
<td>2560*</td>
<td>1280</td>
<td>1280</td>
<td>1280</td>
</tr>
<tr>
<td>ΔJapanese Classical strain</td>
<td>640</td>
<td>640</td>
<td>640</td>
<td>1280</td>
</tr>
<tr>
<td>ΔJapanese 2013 strain</td>
<td>2560</td>
<td>2560</td>
<td>2560</td>
<td>2560</td>
</tr>
<tr>
<td>ΔJapanese INDELS strain</td>
<td>2560</td>
<td>640</td>
<td>1280</td>
<td>2560</td>
</tr>
</tbody>
</table>

*: IFA antibody titer was expressed as the reciprocal of the highest dilution of serum exhibited specific fluorescence

PEDV isolated from affected pigs in 2013-2014 were antigenically similar to each other, and moreover to the CV777 and Japanese classical strain.
PED Vaccine currently used in Japan

There are PED vaccines approved by the Ministry of Agriculture, Forestry and Fisheries (1996).

Mechanism

1. Pregnant sows are intramuscularly inoculated twice before delivery with the live vaccine (monovalent or bivalent).

2. PEDV-specific antibody is induced in the milk of the sows after delivery.

3. Suckling piglets suck continuously the milk containing antibody.

4. Mucous membrane of intestine of suckling piglets is continuously covered with antibody.

5. PEDV which invaded intestine is neutralized by antibody.

Prevention and reduction of diarrhea of suckling pigs

The vaccine can not protect infection of PEDV completely and the expected effect does not appear under poor biosecurity.
Efficacy of PED Vaccine currently used in Japan against the strain isolated in 2013.

Viruses: 1. PEDV 2013 strain isolated from the jejunum of affected pig  
2. Parental strain of PED live vaccine.

Serum: The serum obtained from vaccinated pig  
1. A pig was inoculated twice (at interval of 3 weeks) with live PED vaccine.  
2. The serum was collected at 7 days after second injection.

### Cross-Neutralization test

<table>
<thead>
<tr>
<th>Virus</th>
<th>Miyazaki 2013</th>
<th>Parental strain of vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated pig serum</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Negative control serum</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

Provided by Nippon Institute for Biological Science
Efficacy of PED Vaccine currently used in Japan against the strain isolated in 2013.

Animal: PEDV-negative, SPF pregnant sows

Vaccine: TGE/PED bivalent live vaccine

Challenge virus: PEDV 2013 strain ($10^{6.0}\text{TCID}_{50}$: inoculated into a stomach)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of suckling pig</th>
<th>No. of survivor</th>
<th>Survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant sow A (Vaccinated)</td>
<td>7</td>
<td>5</td>
<td>71.4%</td>
</tr>
<tr>
<td>Pregnant sow B (Vaccinated)</td>
<td>11</td>
<td>8</td>
<td>72.7%</td>
</tr>
<tr>
<td>Pregnant sow (Negative control)</td>
<td>9</td>
<td>2</td>
<td>22.2%</td>
</tr>
</tbody>
</table>

Suckling piglets stay together with their mothers and suck milk freely during experiment.

Provided by [Kaketsuken]
Period of excretion and viremia of PED virus (detection of viral gene)

- Feces: at least 4 weeks
- Nasal swab: at least 3 weeks
- Oral fluid: at least 4 weeks
- Serum: 4 weeks old pig: 3-7 days post-infection
  - Germ-free pig: 30-120 hours post-infection

From: AASV Research Updates 2013, #13-228
Faecal samples were collected every day from 0 (pre) to 22 days post inoculation. PEDV gene was not detected from all samples by conventional RT-PCR (All negative).

Piglets (5 days after birth) without taking colostrum.

**Bioassay of imported spray-dried porcine plasma using piglets**

**Histology & Immunohistochemistry:**
- Small intestine: (antigen detection)
  - HE stain
  - ICH using anti-PEDV serum

**Neutralization test & IFA**
- (antibody detection)

<table>
<thead>
<tr>
<th>No. of piglet</th>
<th>Pre (day 0)</th>
<th>Post (day 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NT</td>
<td>&lt;x2</td>
</tr>
<tr>
<td>IFA</td>
<td>&lt;x10</td>
<td>&lt;x10</td>
</tr>
<tr>
<td>2</td>
<td>NT</td>
<td>&lt;x2</td>
</tr>
<tr>
<td>IFA</td>
<td>&lt;x10</td>
<td>&lt;x10</td>
</tr>
<tr>
<td>3</td>
<td>NT</td>
<td>&lt;x2</td>
</tr>
<tr>
<td>IFA</td>
<td>&lt;x10</td>
<td>&lt;x10</td>
</tr>
</tbody>
</table>

Neutralization test & IFA

**RT-PCR (gene detection)**

Feces were collected every day from 0 (pre) to 22 days post inoculation. PEDV gene was not detected from all samples by conventional RT-PCR (All negative).
Resistance to physical and chemical action of PEDV

**Temperature**  Comparatively stable at 50°C, inactivated at 60 °C for 30min.

**pH**  Stable at pH5-9(4 ℃), pH6.5-7.5(37°C),
inactivated at less than pH4 and more than pH9

**Chemicals /Disinfectants**
Susceptible to the organic solvents, invert soap and other ordinary disinfectants

**Survival**

<table>
<thead>
<tr>
<th>Environment</th>
<th>condition</th>
<th>Period for detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh feces</td>
<td>40°C～60°C</td>
<td>up to 7 days</td>
</tr>
<tr>
<td>Contaminated Feed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet</td>
<td>RT</td>
<td>at least 28 days</td>
</tr>
<tr>
<td>Dry</td>
<td>RT</td>
<td>up to 7 days</td>
</tr>
<tr>
<td>Slurry</td>
<td>4°C, —20°C</td>
<td>at least 28 days</td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>up to 14 days</td>
</tr>
<tr>
<td>Contaminated water</td>
<td>RT</td>
<td>7 days</td>
</tr>
</tbody>
</table>

From ; OIE Technical Factsheet
Possible survivability of PEDV in the affected farm

A follow-up survey at a PED affected farm (farrow-to-finish) was performed in Japan. Feces or rectal swabs were collected from pigs for detecting the gene of PEDV. (Virus isolation has not been carried out.)

<table>
<thead>
<tr>
<th>Months post outbreak</th>
<th>Farrowing piglets</th>
<th>Fattening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30-40 days old</td>
<td>70 days old</td>
</tr>
<tr>
<td>1</td>
<td>1/10*</td>
<td>4/10</td>
</tr>
<tr>
<td>2</td>
<td>2/5</td>
<td>3/10</td>
</tr>
<tr>
<td>4</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>6</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

* No. of PCR positive / No. of samples

In this case...
At 1 month post-outbreak of PED, viral gene has detected from almost all growing stages of pigs. Viral gene has not been detected after 4 months post-outbreak of PED.
Characteristics of PED in Japan (2013-14)

1. Japanese strains of PEDV are genetically close to those recently detected in Asia and American continent in Group II (predominant) and INDELS type in Group I.

2. These new strains of PEDV might have been introduced into Japan from overseas, just before an epidemic. But the routes and forms of incursion were still unknown.
   * The imported, PED PCR positive spray-dried porcine plasma was not the cause of PED. Bioassay using piglets denied its possibility as the origin of PED in Japan.

3. The PEDV may have spread to the whole country through movement and/or shipment of the infected pigs without symptoms (subclinical cases) and through the contaminated people (clothes and boots), vehicle and materials. Cross-contamination has probably occurred with high frequency at common facilities such as slaughter houses and animal markets.
Characteristics of PED in Japan (2013-14)

4. PEDV strains isolated from affected piglets are antigenically identical to the vaccine strain previously developed in Japan. Results of animal experiments demonstrated that currently provided vaccine is effective against the strains isolated in 2013-2014.

5. Diagnostic data of field cases in 1996 and in 2013-14 indicated that there are no differences of symptoms, lesions and tissue tropism of virus between previous cases caused by the strains in Group Ⅰ and 2013 cases caused by the strains in Group Ⅱ. It seems that pathogenicity of INDELS type strains is lower than that of the others (not confirmed).

6. Follow-up surveys of several affected farms suggested that viral gene can be detected from healthy pigs for a long period of time after occurrence of PED.

…………….We need more information to prepare for the next outbreak.
Prevention of the incursion and spread of PEDV

Infected pigs
- Quarantine
- Exchange of clothes and boots
- Washing and disinfection

Contaminated People, materials
- Washing and disinfection

Contaminated Vehicle
- Washing and disinfection

Wild animals and birds (possible carriers of virus)

Prevent the incursion of virus

Prevent infection of pregnant and suckling pigs

Prevent the spread of virus in the farm

Affected pigs:
- Isolation
- Exclusion (all-out)
- Disinfection of the pigsty

Joint FAO/OIE Workshop on Swine Disease Control in Asia
Preventive measures against PED in Japan

MAFF has provided basic policy and guidance on PED control measures

Reinforcement of biosecurity practices
  to prevent introduction of the virus
  disinfection of people, vehicles, equipment and other goods at farm entrance
  to prevent the virus transmission between farms
  disinfection of vehicles on livestock related facilities
  to prevent the spread of the virus within the farms
  appropriate animal waste management

Epidemiological investigation
  to identify the source and route of infection
  to identify any risk factors for introduction and transmission

Promotion of vaccination
  Recommendation of vaccination
  Support of the stable supply and systematic storage by the producer groups

Financial support to PED affected prefectures
  in order to enhance PED biosecurity measures.

Recently, the manual for control and prevention of PED has been distributed.
Thank you very much for your attention